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TEMPORARY IMMUNITY IN ALFALFA ORDINARILY SUSCEPTIBLE TO ATTACK BY THE PEA APHID[1]

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INTRODUCTION

Outbreaks of the pea aphid (*Macrosiphum pisi* (Kalt.)) on alfalfa in the Middle West are sporadic in occurrence. It has been observed that individual plants known to be highly susceptible in the nursery in some seasons will not support an aphid population in other seasons. Aphids placed on such plants after they have been brought into the greenhouse in November failed to live on the stems developed in the field; however, when these plants were cut back to permit new growth they became so heavily infested that they were quickly killed. The intermittent resistance of alfalfa to the pea aphid is referred to in the present paper as temporary immunity. The intent of this paper is to point out the causes of this form of immunity and to indicate the danger of confusing temporary immunity with continuous immunity.

EFFECT OF LIGHT, TEMPERATURE, AND MOISTURE ON RESISTANCE OF ALFALFA TO APHIDS

Davidson (*1*),[3] in discussing the feeding of the bean aphid (*Aphis rumicis* L.) on the English broadbean, or Windsor bean (*Vicia faba* L.), observed that the aphids did not feed so readily during some weeks as during others, and that they fed very little on the older tissue at any time. He also noted that plants under reduced light, although succulent, had small populations of aphids. His suggestions to explain this situation were: (1) The cell sap may be unfavorable for apid development during the period of low reproduction; (2) there is a reduced carbohydrate production by the plant; and (3) the food in the older tissues is unsatisfactory for assimilation. Tottingham stated that during the different seasons in the Temperate Zones the intensity and duration of light radiation received by plants varies greatly, and that at an altitude of 30° the sun delivers only about one-third as much light as at 65°.[4] Miller (*7*) stated that changes in the acidity of plant tissues are associated with diurnal and seasonal fluctuations in light intensity and temperature. He explained that with the absence of light the production of carbohydrates is reduced and sugar is oxidized to form an acid molecular structure.

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[2] The author is indebted to R. T. Cotton, of the Bureau of Entomology and Plant Quarantine, for suggestions and guidance in the preparation of this paper; and to Margaret A. Newcomb, E. C. Miller, J. C. Frazier, Geo. A. Dean, R. C. Smith, and R. H. Painter, of Kansas State College, for criticisms of the work. The photomicrographs were made by F. J. Hanna, also of Kansas State College.

[3] Italic numbers in parentheses refer to Literature Cited, p. 43.

[4] TOTTINGHAM, W. E. PLANT BIOCHEMISTRY. Rev. ed. 2, 249 pp., illus. Minneapolis, Minn. 1937. [Processed.] (See p. 206.)

For about 4 weeks in December and January, when the noonday sun shines more obliquely from the south than in other months, the greenhouse at Manhattan, Kans., where experimental work was conducted, is shaded by the adjoining building so that direct sunlight

FIGURE 1.—Alfalfa seedling that has become dormant while growing in the greenhouse in December and January. *A*, Short internodes of the lateral shoots and the terminal bud. *B*, Apical bud, a potential feeding site for first-instar aphids; natural size.

can reach the plants only from 10 a. m. to 4 p. m. At that time of the year even the most susceptible alfalfa plants became so unattractive and unsuitable to the pea aphid that it was barely able to maintain itself on them. Although moisture and temperature favorable to growth were maintained and the plants remained green, they entered a period of dormancy and, although stem internodes formed, they ceased to elongate (fig. 1). Cross sections of these short internodes when stained with phloroglucinol and hydrochloric acid gave

violet-red and cherry-red colors in the phloem, indicating the presence of lignin and pentose sugar, and when stained with ruthenium red, showed an acid condition (fig. 2, *A*). Alfalfa that is immune to attack under favorable growing conditions stained in this manner in the fourth and fifth internodes.

Experiments in the greenhouse under light and temperature conditions suitable for active growth showed that when the water sup-

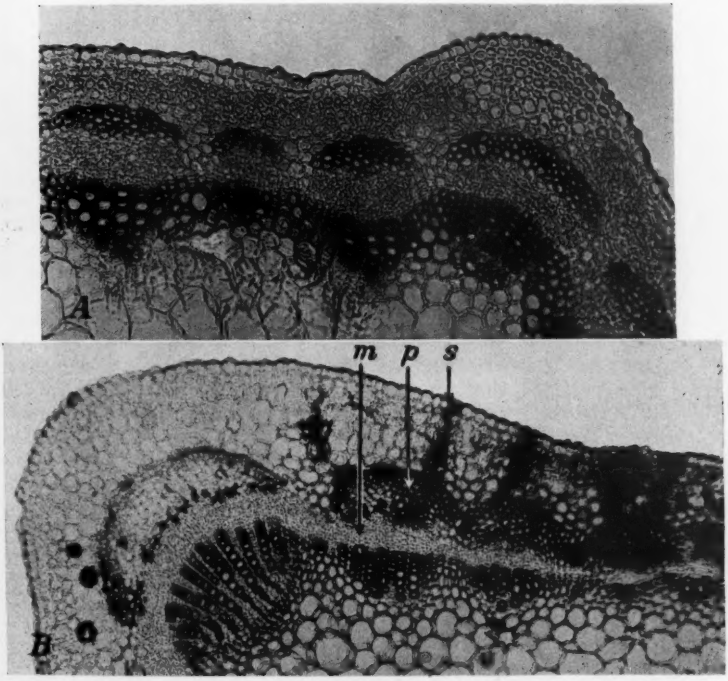

FIGURE 2.—Cross section of stem of dormant alfalfa plant, which is resistant to the pea aphid. *A*, Darker areas show the location of lignin and pentose sugar in the pericycle, outer phloem, and rays. *B*, Area where aphids have been obtaining food in the outer phloem; *b*, Outer phloem; *m*, metaphloem (the stained area ceases abruptly at the edge of the metaphloem); *s*, area penetrated by aphid's setae, stained, × 110.

ply of a plant was deficient for about 2 days the aphids left the feeding place. The outer phloem tissue of a section of a stem grown under these conditions, when given microchemical tests, indicated the presence of lignin, pentose sugar, and an acid condition (fig. 2, *B*). The tissue stained red to violet with phloroglucinol and hydrochloric acid. When the plant was watered, inducing new growth to develop, the aphids returned and fed on the new growth.

In Kansas alfalfa planted in the field in August usually had only a light infestation of *Macrosiphum pisi* by the latter part of September, or during October and November. Observations indicated that a young alfalfa seedling will support an adult aphid on its cotyledons,

the aphid will produce young, and after the plant has developed trifoliate leaves aphids will develop on the apical bud (fig. 1, *B*). In fields of old alfalfa only occasional infestations were present in the fall of the year, depending on the time of cutting in relation to the date of the first frost. Although pea aphid infestations were usually present in the fall, large populations did not develop on either the seedlings or the old plants. According to Peltier and Tysdal (*11*), there was a sharp drop in the moisture content of the tissues of all alfalfa varieties tested by them in October, and it might continue to drop until April. This is the reaction of an alfalfa plant approaching a state of dormancy and resistance to cold, and in this condition it evidently is also resistant to the pea aphid.

In order to determine the biochemical condition of such plants grown in the field, stems were taken and fixed for sectioning on November 3, 1942, from alfalfa plants that had been susceptible to aphid attack in the spring but that did not support an aphid population in the fall. Microchemical tests of the internodes, where aphids would normally feed, indicated the presence of acid substances. Ruthenium red and methylene blue were the indicators used. Other tests with phloroglucinol and with diphenylamine indicated the presence of pentose sugar in the outer phloem. Figure 2 illustrates the reaction to microchemical staining of a dormant alfalfa stem. The plants upon which aphids were feeding in the greenhouse were similarly tested but gave negative reactions to stains for acid and pentose sugar. This fact indicated that the relatively light infestations in the fall were due to unfavorable food conditions resulting from the biochemical reactions of the plant to low temperature and a deficiency of moisture.

It may be inferred from the greenhouse and field observations described above that light, temperature, and moisture, when insufficient to meet the minimum requirements for plant growth, tend to induce dormancy in alfalfa and inhibit the feeding of aphids. Under such conditions the deposition of lignin in the cell walls of the pericycle produces a sclerenchymatous condition which is associated with resistance.

AVAILABILITY OF FOOD IN SUSCEPTIBLE AND RESISTANT ALFALFA PLANTS

It was commonly observed that the aphids reacted differently on individual alfalfa plants growing in the same flat or plot under favorable conditions of temperature, moisture, and light. Aphids were placed on resistant plants growing in the same flat in the greenhouse with susceptible plants. The following day the aphids had left the resistant plants and were found on the susceptible plants. By growing thousands of plants under identical conditions in the greenhouse, it was possible to eliminate the most susceptible ones and at the same time to locate those having high immunity. These observations indicated that the resistance or susceptibility of the alfalfa plant to pea aphid attack might be directly related to the abundance or scarcity of suitable food materials in the plant tissues.

The pea aphid feeds by imbibing its food in liquid form through a tubelike beak. It inserts the stylets or setae of its mouth parts between the cells of the cortex, through the endodermis into the outer phloem. *A*, *B*, and *C*, of figure 3 are cross sections of alfalfa stems

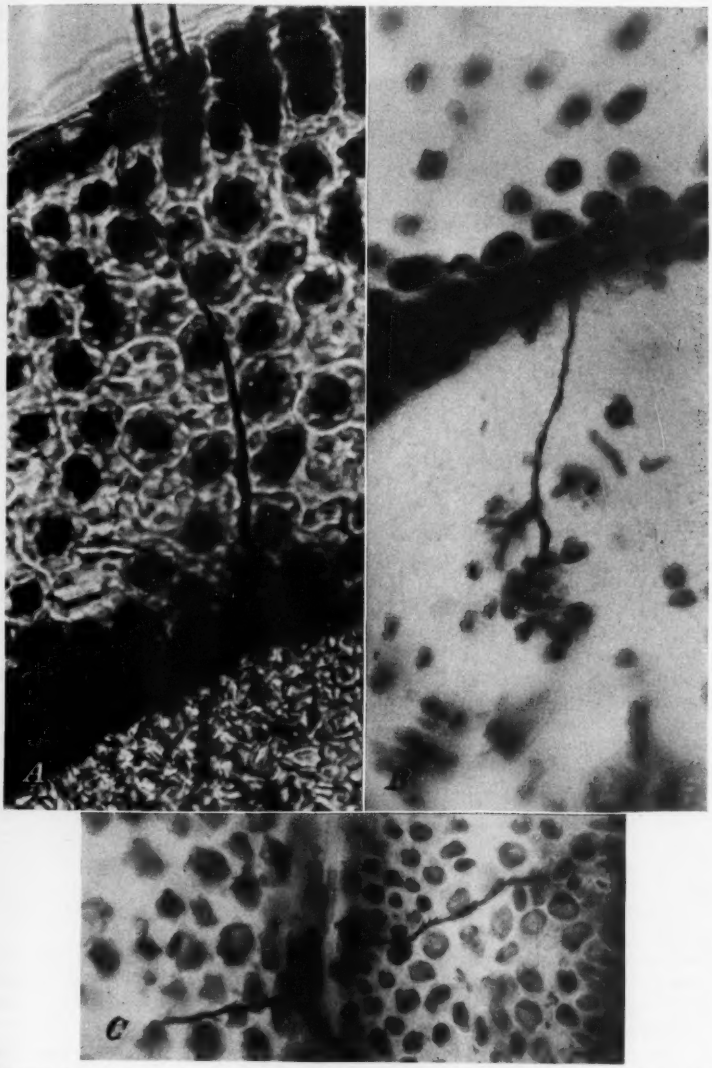

FIGURE 3.—Cross section of an alfalfa stem with setal tube of *Macrosiphum pisi* extending through the cortex and the epidermis and into the region of the outer phloem. *A*, The setal tube appears to branch at the distal end; *B*, lower part of section; *C*, first visible internode of a stem. Note the many barblike projections of the setal tube. Apparently the aphid was penetrating the cell walls in search of food. Aphids did not remain to feed on this internode, which indicates that food was not available. This internode was stained to determine the presence or absence of pectic substances. All × 650.

showing the paths made by the aphid's mouth parts through the tissues. It is believed that the mouth parts of an aphid, like those of some other insects, secrete a fluid which forms a tube, referred to as a setal tube (fig. 3, *C*). Studies of histological sections like those illustrated indicate that *Macrosiphum pisi* obtains its food from the phloem tissue of the alfalfa stem. Judging from the known structure and function of this tissue, it seems likely that *M. pisi* obtains its protein food from the liquid content of the sieve tubes of the phloem. Steele (*12, pp. 288–290*) stated that the main seat of protein and carbohydrate synthesis is in the green leaf and that these materials are transferred through the phloem tissue of the bark to the growing points of the plant, where they are resynthesized for tissue building and storage. It was believed, therefore, that the chemical character of the substances present in the tissue from which *M. pisi* evidently obtains its food might explain its ability or inability to subsist on certain individual plants and on different parts of a single stem.

An attempt to identify the kind of sugar in an alfalfa plant and in the gut of an aphid that had been feeding on it was made by means of a microchemical test for osazones according to a technique developed by S. H. Eckerson, of the Boyce Thompson Institute.[5] For this work phenylhydrazine hydrochloride and sodium acetate in glycerol were used. According to this method the reagents are prepared as follows: Phenylhydrazine hydrochloride is pulverized in a mortar, dissolved in glycerol, 1:10, filtered and kept in a brown bottle. Sodium acetate is dissolved in glycerol, 1:10, and kept in a brown bottle. For osazone formation, a drop of phenylhydrazine hydrochloride and a drop of sodium acetate are mixed on a slide, a section of tissue is placed in the reagent, and a cover glass is added.

Each internode, beginning with the first one visible below the apical bud, was scraped to break the cell walls in the phloem and the sap was pressed into a drop of water on a slide. In order to remove the larger pieces of bark the water containing the sap thus obtained was then run onto another slide and tested for osazones. Figure 4, *A*, shows an osazone produced from sugar in the third visible internode. It is representative of the osazones obtained from the first and the fifth internodes, counting from the top of the stem. Apparently fewer osazones were produced from the fifth internode than from the third or the fourth. The sixth internode did not produce distinct osazones and none were obtained from the seventh or eighth internodes.

The internodes that produced osazones had not completed their growth in length, and these were the ones on which aphids would feed (fig. 5, *A*). Stems of both resistant and susceptible plants produced osazones.

Tests were made for osazones in the hindgut of *Macrosiphum pisi* and bodies resembling the osazones in alfalfa were found (fig. 4, *B*). A comparison of the osazones of sucrose and glucose made from commercial cane and corn sugars, respectively, is shown in figure 4, *C*, and *D*. There was a striking similarity between the sucrose osazones, those produced from sugar in alfalfa, and those found in the hindgut (fig. 4, *B*, *F*) and cornicles (fig. 4, *E*) of the aphid. Tests of

[5] ECKERSON, S. H. MICROCHEMISTRY. 36 pp. Dept. of Botany, Univ. of Chicago. [1920 (?).] [Processed.]

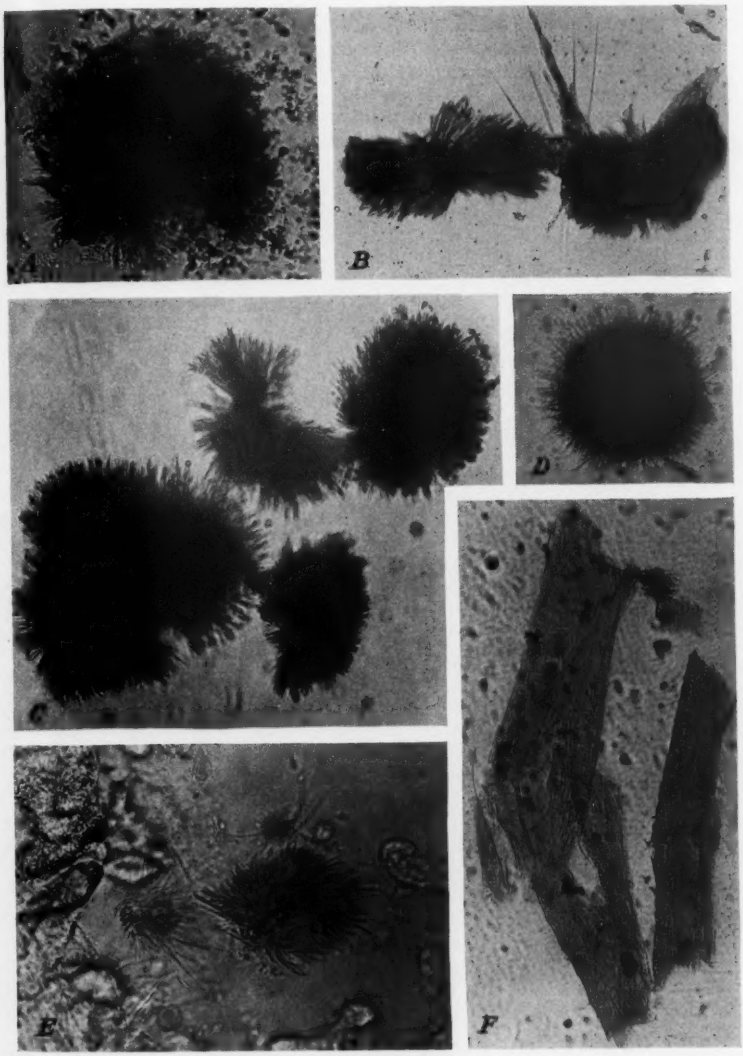

FIGURE 4.—Osazones obtained from an alfalfa stem and from the intestines of an aphid. (Note the individual crystals projecting from the mass.) *A*, Osazone from the third visible internode of a stem. × 650. *B*, Osazones from the hindgut of *Macrosiphum pisi*, × 650. *C*, Sucrose osazones made from commercial cane sugar, × 650. *D*, Glucose osazone made from commercial glucose. The crystals are much finer than those from sucrose, × 650. *E*, Osazones from the base of a cornicle of *M. pisi*, × 650. *F*, Section of hindgut of *M. pisi* showing osazones, × 110.

pea vine and a leaf upon which *M. pisi* had fed showed osazones resembling those from sucrose. The same type was produced in Austrian field peas, a host of the pea aphid. Osazones resembling those from glucose were not obtained from the aphids or from alfalfa. First-instar aphids just beginning to feed yielded no osazones when tested, but as they neared completion of the first instar when feeding on the

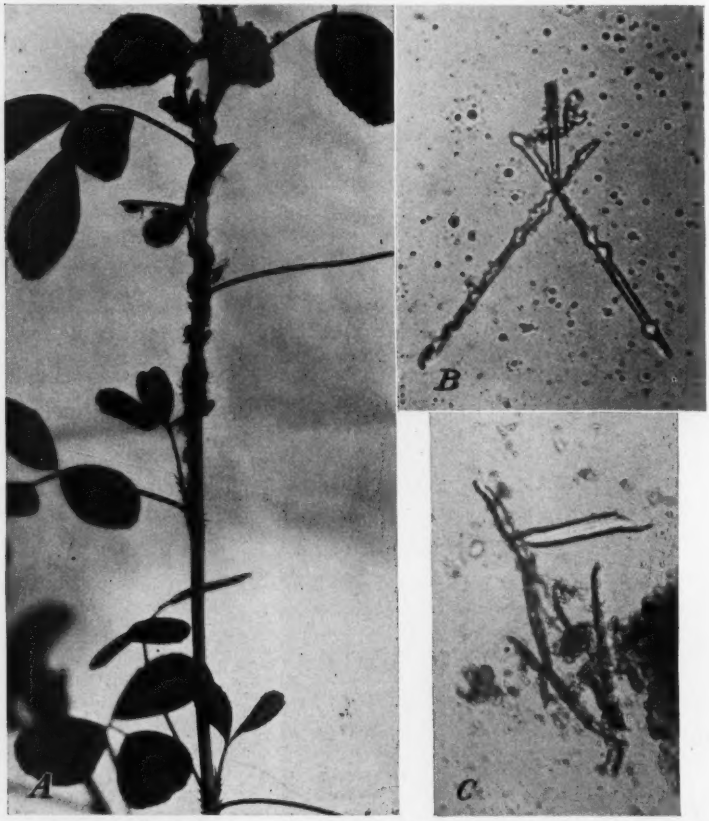

FIGURE 5.—*A*, Alfalfa stem, showing the distribution of aphids. The first and fifth internodes have no aphids on them, natural size. *B*, Osazones obtained from a first-instar *Macrosiphum pisi*, found feeding on an alfalfa steam, × 110. *C*, Osazones obtained from a mature alfalfa leaf, × 110.

stem it was possible to obtain osazones from the guts, as shown in figure 5, *B*.

In repeated tests of the apical leaf (covered with small aphids, as shown in figure 1, *B*) few or no osazones were found. An aphid larger than the second instar was seldom observed feeding on the apical leaf. Adult aphids were more often found on stems that produced osazones abundantly, that is, on internodes 2, 3, and 4 in alfalfa. Aphids were seldom seen feeding on a mature leaf. Mature

alfalfa leaves tested in this experiment usually gave negative reactions for osazones. Figure 5, *C*, shows a simple type of osazone obtained from a mature leaf. These osazones resemble those obtained from first-instar aphids.

Mumford and Hey (*8*) pointed out that a highly nitrogenous diet stimulates reproduction in insects, and some workers feel that immunity or susceptibility to insect attack is closely related to the protein value in the plant. According to Miller (*7*) and Meyer and Anderson (*6, p. 451*), protein synthesis is dependent on the "carbohydrate-nitrogen ratio." Since resistant plants produced sucrose in the stems, as did susceptible plants, the "carbohydrate-nitrogen ratio" would not be changed so far as protein synthesis is concerned.

Plant physiologists state that the apical bud during its formative period and early growth receives its protein and carbohydrates from other parts (leaves and stems) of the plant. At this period the "cell sap" has attained its highest proportion in the growing protoplast. As the internodes elongate the cell walls require additional carbohydrates for building material. Aphids feed on the apical bud only during the first instar. Tests of the apical bud show few or no sucrose osazones and, since larger aphids seek feeding areas where sucrose is abundant, it would seem that the availability of sucrose is the probable explanation for their shift to other feeding places, and that protein is not a factor of resistance in this case.

Dunnam and Clark (*2*) have reported a larger total number of young per aphid observed on cotton plants dusted with calcium arsenate than on plants not dusted. They observed that the acidity of the soil was reduced where the plants had been dusted with calcium arsenate.

In a controlled experiment, the writer limed one part of an alfalfa plot and left the rest untreated. Sweepings of the alfalfa on May 18, May 30, and June 23 showed twice as many aphids on the plants growing on the limed part of the plot as on the unlimed part. In a field near Manhattan, Kans., the heavy outbreak of the pea aphid in 1943 caused the death of the alfalfa tops in well-defined areas or spots. Soil samples taken from these areas in 1944 showed a pH value of 6.28, whereas samples of the soil taken 100 yards away from an area where no severe plant injury occurred showed a pH value of 5.86. Population counts in 1944, as determined by the number of aphids in 20 sweeps of an insect net, showed the population of aphids to be four times as great on an area with a soil having a pH value of 6.28 as on the other parts of the field where the soil pH was lower. These two observations indicate that aphid populations tend to be higher on plants growing in soil with a high pH value, or with additional available calcium. Parker and Truog (*10*) state that the greater part of the calcium taken up by the plant is probably used for the neutralization and precipitation of the acids in the plant sap.

SOME PLANT PROCESSES THAT AFFECT THE FEEDING OF APHIDS

Gortner (*4*) and Gisvold and Rogers [6] point out that pectic substances are abundant in newly forming tissue. These substances include pectic acid, pectin (which serves to cement and support new tissue), pentosans, and pentose sugar, all of which aid in the main-

[6] Gisvold, O., and Rogers, C. H. THE CHEMISTRY OF PLANT CONSTITUENTS. Rev. ed., 392 pp. Minneapolis, Minn. 1941. [Processed.]

tenance of the water balance of the plant and precede the synthesis of other carbohydrates. According to Miller (*7, p. 19*), "The formation of pectic substances in plants appears to occur when metabolism and growth are at their maxima. As growth slows and as maturity is reached the production of pectic materials decreases and those present in the [cell] wall are slowly converted into other substances." In this process it is believed that lignin is synthesized from substances in the structures containing hemicellulose. The presence of lignin in the pericycle of an internode is an indication that the stem is resistant to the pea aphid at that internode (fig.2, *A*).

Norman (*9*) stated that the hexose → pentose transformation through the intermediate formation of uronic acid does not appear to be completed in the mannose series. However, a polygalacturonic acid containing some sugar units develops as pectin. It is during the period in which the hexose → pentose transformation takes place in the alfalfa plant that the condition of immunity develops. It has been observed that the first visible internode of a susceptible alfalfa plant growing under favorable conditions is not fed upon by the pea aphid. Microchemical tests of both susceptible and resistant plants revealed the presence of pectic substances in this internode. The second, third, and fourth internodes, where aphids normally feed on a susceptible plant, gave negative reactions for pectic substances and acidity, whereas the corresponding internodes of a resistant plant gave positive reactions. These results indicate that resistance in alfalfa is dependent on the character of the carbohydrates present and on the acidity of the sap. Plant chemists have shown that the acidity of the cell sap varies with differences in temperature, light, and moisture. In the tests performed in this study the continuously resistant plants maintained an acid condition throughout their growth, which prevented feeding by aphids.

According to Meyer and Anderson (*6, pp. 473–474*), there appears to be an optimum hydrogen-ion concentration for the activity of each enzyme, as well as upper and lower limits of hydrogen-ion concentration, beyond which the enzyme is inactive, or may actually be destroyed. Tests by Fife and Frampton (*3*) of the pH gradient in leaves of the sugar beet showed that the success of the beet leafhopper (*Eutettix tenellus*) in finding the phloem in the petiole is related to the hydrogen-ion concentration. They stated that the leafhopper prefers an alkaline food (pH 8.5) to one that is acid (pH 5.0) in reaction. In a study of cell-sap acidity and the incidence of a white fly, *Bemisia gossypiperda*, on cotton, Husain, Puri, and Trehan (*5*) showed that (1) there is a difference in the hydrogen-ion concentration of varieties of cotton plants, (2) the cell-sap acidity of the same plant differs with a change of seasons, (3) the pH gradient from top to bottom of the plant varies with the age of the plant, and (4) the attack of the white fly was less severe when the hydrogen-ion concentration was relatively low. From the results reported by these workers, and by the present author in this article, it seems logical to infer that the enzyme activity of the salivary secretion of the aphid is inhibited by the acid condition of the cell sap, and as a result the food in the cells cannot be digested.

Breeding tests have shown that resistant plants when cross-pollinated produce resistant progeny.

SUMMARY AND CONCLUSIONS

Temporary immunity to the feeding of the pea aphid (*Macrosiphum pisi*) on alfalfa is associated with an acid condition in the plant. The acidity may be caused by (1) a deficiency of water, (2) a deficiency of light for photosynthesis, (3) temperatures too low for rapid growth of alfalfa, and (4) temperatures sufficiently high to cause the formation of lignin.

Tests of the cell sap for osazones have shown that aphids feed most in the elongating internodes, where growth is rapid and where sucrose is most abundant. Since the synthesis of protein is dependent on a "carbohydrate-nitrogen ratio," it is believed that resistance of alfalfa to the pea aphid is correlated primarily with an acid condition and a scarcity or absence of sucrose, rather than with a scarcity of proteins in the plants.

LITERATURE CITED

(1) DAVIDSON, J.
1925. BIOLOGICAL STUDIES OF APHIS RUMICIS LINN. FACTORS AFFECTING THE INFESTATION OF VICIA FABA WITH APHIS RUMICIS. Ann. Appl. Biol. 12: 472–507, illus.

(2) DUNNAM, E. W., and CLARK, J. C.
1941. COTTON APHID MULTIPLICATION FOLLOWING TREATMENT WITH CALCIUM ARSENATE. Jour. Econ. Ent. 34: 587–588.

(3) FIFE, J. M., and FRAMPTON, V. L.
1936. THE PH GRADIENT EXTENDING FROM THE PHLOEM INTO THE PARENCHYMA OF THE SUGAR BEET AND ITS RELATION TO THE FEEDING BEHAVIOR OF EUTETTIX TENELLUS. Jour. Agr. Res. 53: 581–593, illus.

(4) GORTNER, R. A.
1938. OUTLINES OF BIOCHEMISTRY . . . Ed. 2, 1017 pp., illus. New York and London.

(5) HUSAIN, M. A., PURI, A. N., and TREHAN, K. N.
1936. CELL SAP ACIDITY AND THE INCIDENCE OF WHITEFLY (BEMESIA GOSSYPIPERDA) ON COTTONS. Current Sci. [India] 4: 486–487.

(6) MEYER, B. S., and ANDERSON, D. B.
1939. PLANT PHYSIOLOGY. 696 pp., illus. New York.

(7) MILLER, E. C.
1938. PLANT PHYSIOLOGY . . . Ed. 12, 1201 pp., illus. New York and London.

(8) MUMFORD, E. P., and HEY, D. H.
1930. THE WATER BALANCE OF PLANTS AS A FACTOR IN THEIR RESISTANCE TO INSECT PESTS. Nature [London] 125: 411–412.

(9) NORMAN, A. G.
1937. THE BIOCHEMISTRY OF CELLULOSE, THE POLYURONIDES, LIGNIN, ETC. 232 pp., illus. Oxford.

(10) PARKER, F. W., and TRUOG, W. E.
1920. THE RELATION BETWEEN THE CALCIUM AND THE NITROGEN CONTENT OF PLANTS AND THE FUNCTION OF CALCIUM. Soil Sci., 10: 49–56, illus.

(11) PELTIER, G. L., and TYSDAL, H. M.
1931. HARDINESS STUDIES WITH 2-YEAR-OLD ALFALFA PLANTS. Jour. Agr. Res. 43: 931–955, illus.

(12) STEELE, C. C.
1934. AN INTRODUCTION TO PLANT BIOCHEMISTRY. 356 pp., illus. London.

YELLOW WILT OF SUGAR BEET IN ARGENTINA [1]

By C. W. BENNETT, *senior pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture,* and CARLOS MUNCK, *agronomist, Argentine Southern Railway* [2]

INTRODUCTION

In 1939 Huyskes (*4*) [3] described two types of symptoms, which he attributed to adverse soil-moisture conditions, on diseased sugar beets (*Beta vulgaris* L.) in the valley of the Río Negro in Argentina. The symptoms, however, are not those that ordinarily result from deficiency or excess of water, and additional evidence now available indicates that the trouble is not due to water relations or to other environmental conditions such as soil deficiencies, salt accumulation, or climatic factors.

In Argentina, the disease was studied by the junior author from 1938 through 1941, and in January and February 1941 it was observed by the senior author, who later made studies of diseased material brought from Argentina and placed in the quarantine greenhouses at Arlington, Va. The results of these joint studies are reported here.

HISTORY OF THE DISEASE

Sugar beets have been grown experimentally in a number of localities in Argentina to test the crop for suitability as an additional source of sugar in that country. In two localities factories were constructed and attempts made to establish a beet-sugar industry.

The first factory began operations in 1929 in the valley of the lower Río Negro about 80 miles from the Atlantic coast. This area has a fertile soil, an abundant supply of water for irrigation, and a favorable climate for production of sugar beets. From the beginning, however, yields were low and varied considerably from year to year, as shown by the yield data (table 1) taken from the records of the factory of the Compañia Industrial Agrícola "San Lorenzo" Lda. It is now reasonably certain that these low fluctuating yields were due largely to a disease that caused yellowing, wilting, and complete collapse of the plants usually after they were well advanced toward maturity. Because of these characteristic symptoms, the disease is here designated "yellow wilt."

[1] Received for publication January 7, 1944.

[2] The authors are greatly indebted to G. H. Coons, principal pathologist, Division of Sugar Plant Investigations, for helpful suggestions during the course of the studies here presented and for making many of the notes on diseased plants in the studies conducted in the quarantine greenhouses at the Arlington Experiment Farm, Arlington, Va.

[3] Italic numbers in parentheses refer to Literature Cited, p. 64.

The origin of yellow wilt is unknown, but there is evidence that it occurred in the Territory of Río Negro prior to the establishment of the factory, since Henderson (*2*) reported that a disease that caused sudden wilting and death of plants destroyed all the experimental plantings of sugar beets at the Experiment Station of Río Negro, near General Roca, in 1926–27.

In 1937–38 and 1938–39 yellow wilt reached a peak of severity in the plantings of the Río Negro Valley and was responsible for almost complete loss of the crop. It caused serious injury also in small plantings in the valley of the Río Colorado about 90 miles to the north. In 1939–40 and 1940–41 sugar beets were grown in Río Negro only on an experimental scale, and in 1941 sugar-beet culture in this area was discontinued.

A second factory was built in the Province of San Juan and abandoned a few years later. Little information is available regarding the cause for the discontinuance of this factory, but it seems probable that a disease problem contributed to its failure.

TABLE 1.—*Sugar-beet yields on the land of the Compañia Industrial Agrícola "San Lorenzo" Lda. in the lower Río Negro Valley of Argentina, 1928–40*

Season	Area cropped to sugar beets		Total yield	Yield per hectare
	Planted	Harvested		
	Hectares	*Hectares*	*Kilograms*	*Metric tons*
1928–29			500,000	
1929–30	311		2,879,600	9.2
1930–31	436		1,008,180	2.3
1931–32	405	403	3,406,230	8.4
1932–33	362	357	3,533,280	9.9
1933–34	222	205	3,204,070	15.6
1934–35	199	189	1,207,140	6.3
1935–36	193	175	2,625,395	15.0
1936–37	205	199	1,119,685	5.6
1937–38	297	265	815,909	3.0
1938–39				
1939–40	52	46	159,860	3.4

HOST RANGE

In the valley of the Río Negro yellow wilt has been important chiefly as a disease of sugar beets. Limited evidence indicates that it is capable of causing severe injury also on red garden beets (*Beta vulgaris*) and Swiss chard (*B. vulgaris* var. *cicla* L.). Red garden beets found in fields of sugar beets, and other plants that may have been hybrids between these and sugar beets, appeared to be as susceptible to the disease as the adjacent sugar beets. Late in December 1940, one short row of Swiss chard in a garden near plantings of sugar beets began to be severely affected by what appeared to be yellow wilt, and by the early part of the following February all the plants were either severely affected or dead.

An unidentified species of *Chenopodium* and *C. album* L., both growing more or less abundantly in fields of diseased sugar beets, showed no evidence of infection; nor were plantings of potatoes (*Solanum tuberosum* L.) and tomatoes (*Lycopersicon esculentum* Mill.) in the vicinity of diseased beet plantings affected.

SYMPTOMS

SYMPTOMS IN THE FIELD

In 1940–41 yellow wilt was very severe in plantings of sugar beets near Conesa in the lower Río Negro Valley. The almost complete destruction of the early plantings is illustrated in figure 1. As in the two previous seasons, two rather distinct types of symptoms were produced. One type was characterized by yellowing and stunting

FIGURE 1.—Field of sugar beets in the lower Río Negro Valley, almost completely destroyed by yellow wilt during 1940–41.

of the plants and the other by rapid wilting of apparently normal plants, often followed by death within a few days. The conditions characterized by the two types of symptoms are designated as the "yellows phase" and the "wilt phase" of the disease, respectively.

YELLOWS PHASE

In 1940 yellowed plants were first observed in plantings in the lower Río Negro Valley about the middle of November. In mid-January 1941 most of the diseased plants were stunted and yellow. The leaves appeared to be somewhat thickened and brittle and were usually rolled or partly folded inward. Some of the outer leaves were dead, and others were scorched along the edges and between the larger veins. The inner leaves had a tendency to assume a position approaching the vertical (fig. 2, *A*), and the edges of the blades were wavy and often irregular.

FIGURE 2.—*A*, Sugar-beet plants showing the yellows phase of yellow wilt. Leaves are yellow, are usually rolled, brittle, and necrotic, and have a tendency to assume a vertical position. *B*, Healthy (*a*), wilted (*b*), and yellowed (*c*) plants. Note the beginning of small shoots from axillary buds in *c*. *C*, Plants showing early stages of the wilt phase.

On the half-grown and larger leaves of some of the plants, the larger veins and some of the smaller ones were so yellow that the vein pattern was clearly evident. In a few instances this vein yellowing was restricted to the leaves on one side of the plant and sometimes to one side or to the basal portion of one or more leaves. The yellowed areas were somewhat broader than the veins themselves. Yellowing was not in all cases continuous along the veins but sometimes occurred in broken lines. On the majority of affected plants, however, vein yellowing was not conspicuous. No evidence of vein clearing or yellowing was observed on the very young leaves of affected plants.

Many of the yellowed plants were still in reasonably good condition the early part of February, and those that continued to grow showed a tendency toward stimulation of growth of axillary buds. The development of axillary buds was not great in any case, however, and the main shoot continued to be dominant. Some of the plants had a slightly rosetted or bunchy appearance.

The roots of diseased plants were smaller than those of healthy plants and were tough and woody. In some cases the tip of the main root died and lateral roots developed. In other instances the tips were flaccid or had begun to dry and rot. In a few instances no external evidence of root deterioration was observed. In all cases there were many dead rootlets, and in most cases there was evidence, from the decidedly tufted appearance of the lines of sutures along which rootlets had been produced, that successive growths of rootlets had been killed.

Besides being woody, the roots of diseased plants, when sectioned transversely or longitudinally, showed dark flecks in the region of the vascular bundles. In more advanced stages of the disease the amount of discolored tissue was much greater. There was no evidence that the phloem was affected more than other tissues. In the earlier stages of injury darkened flecks were noted in the parenchyma just outside the vascular bundles. It is uncertain how much of the discoloration of the tissues in the later stages of disease was due to the disease and how much was caused by secondary agents.

Wilt Phase

In addition to the complex of symptoms just described, a second type of disturbance began to appear conspicuously about the second week in January, and thereafter few of the plants that became diseased developed the type of symptoms that were fully characteristic of the yellowed plants observed earlier in the season

The plants that became diseased after the middle of January were characterized principally by rapid wilting, scorching of mature leaves, and death. Usually large plants with roots 1 to 3 inches in diameter were affected. Frequently large vigorous plants began to wilt about midday, and by late afternoon the leaves had drooped markedly, as shown in figure 2, *C*. The plants recovered somewhat during the night, but the following day they usually wilted so much that the larger leaves lay flat on the ground and did not again fully recover turgidity. By the third or fourth day the older leaves began to burn

and dry, and within a week most of the mature leaves were dead or nearly so. The rapid progress of this phase of the disease is illustrated in figure 3.

In some affected plants, the small central leaves recovered somewhat after the death of the older leaves and again assumed an up-

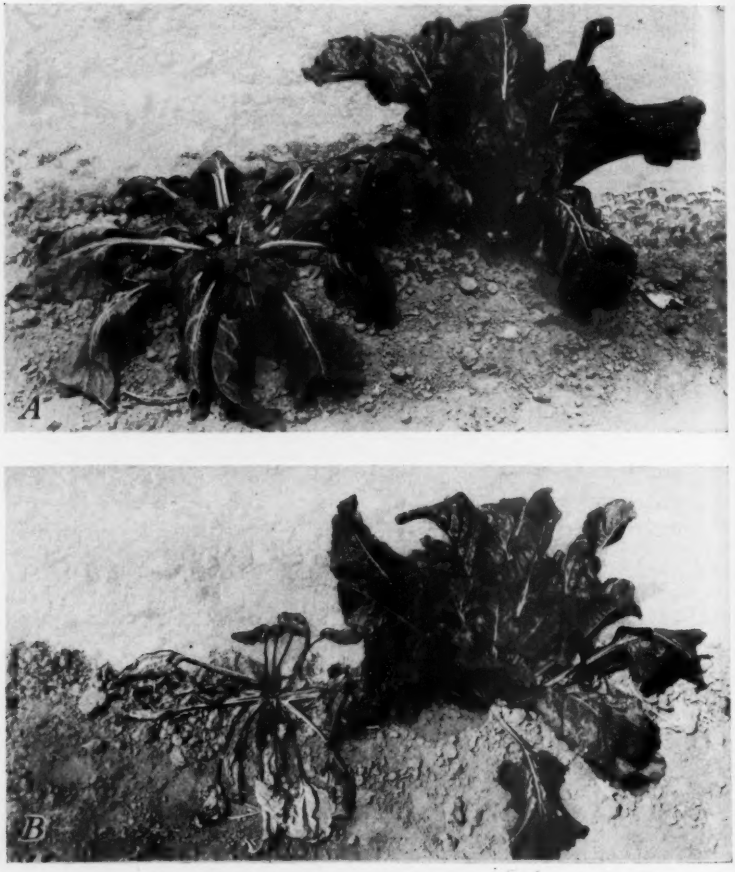

FIGURE 3.—*A*, Sugar-beet plant (at left) showing early stage of wilt, photographed January 20, 1941; *B*, same plant 7 days later.

right position. In such plants, some additional growth was produced but the leaves were thick, folded, and greenish yellow sometimes slightly tinged with purple. Such yellowish tufts of leaves lived until root disintegration was well advanced. In many cases, however, the wilted plants died without producing any appreciable additional growth (fig. 4).

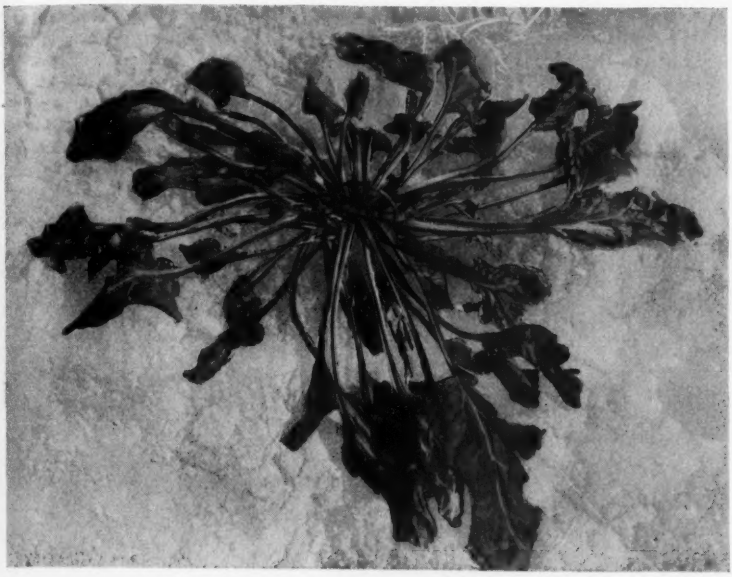

FIGURE 4.—Sugar-beet plant in advanced stage of wilt. This plant showed very little recovery of central leaves and was nearly dead 2 weeks after it showed first symptoms of disease.

Plants dug when the tops displayed the first symptoms of wilt showed no evidence of injury in any part of the main root. However, many of the rootlets were dead or beginning to turn brown, but in all cases some appeared to be normal. There was no evidence of an abnormally large number of rootlets, such as was found on the yellowed plants.

In later stages of wilting, damage to the root was clearly evident. Three or four days after the tops started to wilt, the tips of the roots became flaccid and began to shrivel; and decay soon developed. In this stage the top part of the root was still turgid, but the tissue cut easily with a sharp knife and had lost much of its crispness. The wilting and shriveling, which began in the distal end of the root, gradually extended in the direction of the crown until the entire root was involved. This process sometimes required no more than 1 to 2 weeks. In the later stages, the beets were so shrunken that they could easily be pulled out of the soil, leaving the holes they had formerly occupied clearly outlined. No well-defined discoloration or necrosis of the root tissue could be found prior to the beginning of wilting and shriveling.

In some of the wilted plants the progress of the disease was not so rapid as that just described. Outer leaves wilted, dropped to the ground, and died. The remaining leaves became more upright in appearance and turned yellow or yellowish green. Such plants continued to grow slowly, and there was indication that they might

live for considerable periods. In general appearance they were more or less intermediate between plants showing typical yellows phase and those showing typical wilt phase. In some of the plants of this type a small amount of development of lateral buds was noted, and in a few plants a type of vein yellowing was observed.

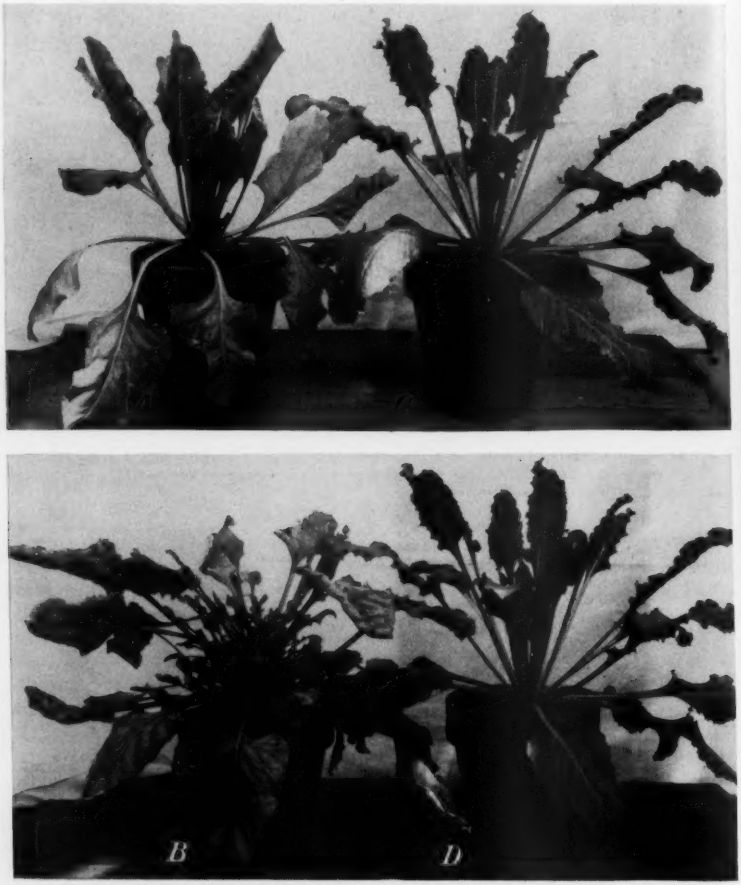

FIGURE 5.—*A*, Sugar-beet plant inoculated by grafting with a plug from a wilted plant, placed in a pot in the greenhouse March 22, and photographed June 12, 1941; the plant shows general yellowing of foliage and early stage of development of shoots from axillary buds. *B*, Sugar-beet plant showing advanced stage of yellow wilt under greenhouse conditions; the central shoot has lost its dominance, and the shoots from axillary buds have developed into a type of witches'-broom; the older leaves are yellow and necrotic. *C* and *D*, Healthy control plants.

SYMPTOMS UNDER GREENHOUSE CONDITIONS

Diseased plants grown in the greenhouse probably showed symptoms more nearly typical of those that the disease is capable of producing under conditions most favorable for development of infected plants.

The first evidence of disease was the stimulation of growth in the axillary buds. These buds soon developed into small shoots having many small leaves with relatively long petioles and short, malformed, yellow blades. The central shoot also produced small, malformed, yellow leaves, and soon the older leaves began to take on a more or less leathery appearance and turn yellow. Figure 5, *A*, shows an early stage of the disease, and figure 5, *B*, a more advanced stage.

Yellowing of veins and leaf malformation (fig. 6) were much more common on plants in the greenhouse than on those in the field. As in the field, some leaves of greenhouse plants were found in which the vein yellowing was present in only part of the leaf area.

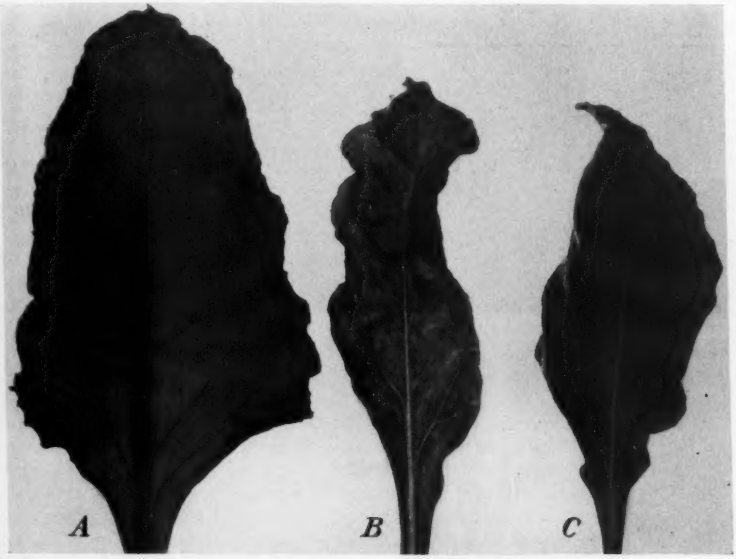

FIGURE 6.—*A*, Leaf from healthy sugar-beet plant; *B* and *C*, leaves from plants affected by yellow wilt, showing types of malformation and clearly outlined vein patterns.

The wilt phase of the disease did not develop in the greenhouse; plants selected in the field as showing early stages of wilt and those infected by grafts from such plants produced only the yellows phase of the disease.

TRANSMISSION OF THE DISEASE

There is considerable evidence that yellow wilt is not transmitted through the soil or by direct contact between diseased and healthy plants in the field or in the greenhouse. In the field many cases were observed where, in thinning, two sugar beets had been left in close contact; in a number of instances it was found that one of these plants was diseased whereas the other was healthy and continued to be free of disease for at least 4 weeks.

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In further tests, diseased sugar-beet plants from the field were placed in pots in the greenhouse and at the same time healthy seedlings were placed in each pot and left to grow along with the diseased

Figure 7.—*A*, Sugar-beet plants still healthy after being grown for 2 months in pots with diseased plants; *B*, sugar-beet plant infected by the yellow wilt virus by means of a graft; *C*, healthy control.

individuals. Figure 7, *A*, shows two such healthy plants after they had grown in close contact with diseased plants for more than 2 months. Several of these seedling plants were kept in pots with

diseased sugar beets for more than 4 months, and all remained healthy.

TRANSMISSION BY GRAFT

Two methods of inducing union between diseased and healthy tissue were used in attempts to transmit yellow wilt by graft. In the first method, plugs of tissue about 2 cm. long and 8 mm. in diameter were removed from the root of diseased plants by means of a cork borer. These plugs were inserted into holes made in the crowns of healthy beets by a cork borer one size smaller than that used for removing the plugs from the diseased root. In the second method, V-shaped slabs were removed from the crowns of diseased roots and inserted in slits made in the crowns of healthy roots. The infected tissue was then bound firmly in place with strips of cloth. In some cases, the grafted beets were placed in sphagnum moss and subjected to low temperatures for a period before being potted; in other cases the grafted roots were potted immediately after they were grafted.

In the first test, plants showing no symptoms of disease were selected in the fields near Conesa, Argentina, February 3, 1941, and taken to Buenos Aires. Ten of these plants were grafted by means of plugs from diseased plants that showed the yellows phase of the disease; 11 were grafted by means of plugs from roots that showed the wilt phase of the disease; and 9 were held as checks. The roots were placed in sawdust in cold storage until March 21, when they arrived in New York, N. Y. They were then taken to Arlington, Va., and transplanted to pots in quarantine greenhouses.

Since these plants were selected in fields where the disease was prevalent, it was expected that some of the plants retained as checks might show disease. Of the 9 check plants, 3 showed disease from the beginning of growth after being transplanted; the 6 other plants remained healthy until discarded several months later. Of the 21 plants inoculated by graft, 11 became diseased, and 10 remained healthy. Of those that became diseased, 2 showed disease from the beginning of growth, possibly indicating that they were infected in the field, and 9 first produced normal leaves and later developed disease. One of these plants showed first symptoms on July 30, nearly 6 months after it was grafted and 4 months after it was placed in the greenhouse. Of the 11 plants that became diseased, the plug of diseased tissue was alive in 9 and dead in 2 on June 9; of the 10 that failed to develop disease, the plug was dead in 9 and alive in 1. These results indicate that the disease was transmitted by the grafts in most of the cases where the grafts were alive on June 9.

There were no differences in symptoms on the diseased plants of the three groups. The wilt phase of the disease did not appear, and the production of axillary shoots was characteristic and more pronounced than under field conditions.

With diseased sugar beets from Argentina, as well as beets infected by graft, as sources of inoculum, nine other tests for graft transmission of yellow wilt were made with plants grown in the United States. The results of these tests are shown in table 2, and one of the plants infected by graft is shown in figure 7, *B*. In eight of the nine tests, transmission was obtained. No infection was observed in any of the check plants. Although infection was relatively low in most of the tests, probably

owing to the death of the grafts before adequate union with healthy tissue occurred, the results demonstrate conclusively that the disease is graft-transmissible.

TABLE 2.—*Transmission of yellow wilt of sugar beets by grafting diseased material to healthy plants*

Experiment No.	Total grafted plants	Grafted plants infected	Total check plants	Check plants infected
	Number	*Number*	*Number*	*Number*
1 [1]	21	11	9	3
2	17	6	17	0
3	16	4	6	0
4	16	2		
5	12	0	10	0
6	11	1	10	0
7	7	2	5	0
8	6	2	6	0
9	5	1	4	0
10	10	7	10	0

[1] All plants used in experiment 1, including check plants, were obtained from the field in Argentina and appeared healthy when selected.

JUICE INOCULATIONS

Attempts were made to transmit the virus of yellow wilt to several kinds of plants under greenhouse conditions. Juice was pressed from tops and from roots of badly diseased plants, and inoculations were made by rubbing the extracted juice over the surface of leaves of healthy plants, with and without an abrasive. In other tests, inoculations were made by introducing juice directly into the region of the growing points of plants by means of a fine needle. Inoculations were made to Turkish tobacco (*Nicotiana tabacum* L.), *N. glutinosa* L., petunia (× *Petunia hybrida* Vilm.), the Scotia variety of bean (*Phaseolus vulgaris* L.), and cucumber (*Cucumis sativus* L.), as well as to sugar beet. No infection was obtained on any of these plants.

TRANSMISSION BY DODDER

Two species of dodder (*Cuscuta subinclusa* Dur. and Hilg. and *C. campestris* Yuncker) were used in tests of ability of dodder to transmit the virus of yellow wilt. Virus-free dodder was trained on diseased sugar-beet plants, and after it had become established stems were trained from diseased to healthy plants. After the dodder was well established on the healthy plants, dodder contact between diseased and healthy plants was broken.

From the results of these tests (table 3) it seems evident that the causal agent was transmitted from diseased to healthy plants in a small percentage of the cases by each species of dodder. The relatively low percentage of infection obtained may be due in part to poor contact by dodder on some of the inoculated plants; but it is probable that a low percentage of infection would be obtained, even under optimum conditions, from dodder contact, since the symptoms and other characteristics of yellow wilt indicate that the causal virus may be more closely associated with the phloem than with the parenchyma and since there is no evidence that either species of dodder is a host of the virus. No explanation is available for the one case of disease in the check plants inoculated by means of supposedly virus-free dodder.

TABLE 3.—*Transmission of yellow wilt of sugar beets by means of dodder*

Species of dodder	Test No.	Results with dodder from infected beet plants		Results with dodder from healthy beet plants	
		Plants inoculated	Plants infected	Plants inoculated	Plants infected
		Number	*Number*	*Number*	*Number*
Cuscuta subinclusa	1	10	0	10	0
	2	9	1	10	0
	3	10	2	10	1
	4	5	0	5	0
	5	10	3	10	0
Cuscuta campestris	6	10	3	10	0
	7	7	1	7	0

TESTS WITH INSECTS

In 1939–40 preliminary tests were made in beet fields near Conesa, Argentina, to determine whether plants protected from insects by cages would remain free of disease and whether introduction of insects into the cages later in the season would result in infection.

For these tests 21 cages covered with close-woven muslin were divided into 3 groups of 7 cages each and placed over rows of sugar beets just after sowing. The cages of group 1 were used to test insects introduced later in the season from diseased plants. The cages of group 2 were raised about 10 cm. above the soil surface the middle of November in order to allow insects free access. The cages of group 3 were used as controls and remained closed during the entire season. Except for 1 cage of group 1 in which the seeds did not germinate, each cage contained from 5 to 7 plants.

By January 4, the beets in the cages had reached considerable size and were healthy although the disease had become general in the fields in which the cages were located. On that date, undetermined numbers of insects were captured on diseased beets in the surrounding fields and introduced into the cages of group 1. These collections consisted of all insects that were captured by sweeping with a net and included several species of leafhoppers.

The first symptoms of disease were noted on plants in the cages of group 1 on February 8, 35 days after the introduction of insects. These symptoms consisted of yellowing and drooping of leaves followed in most cases by necrosis and wilting of the entire plant. The incidence of disease increased, and by March 9 diseased plants were present in 5 of the 6 cages into which insects were introduced. Of the 31 plants in the 5 cages in which infection was obtained, 25 eventually became diseased.

Disease appeared in 4 of the 7 cages of group 2. The first symptoms were noted on December 18, about 30 days after the cages were raised. A total of 17 of the 44 plants in this group eventually became infected.

All the plants in the 7 cages of group 3 remained healthy.

Since these preliminary tests indicated clearly that insects are involved in the spread of the disease under field conditions, further tests were made in 1940–41 in an effort to determine the insect or insects responsible. As in the previous season, cages were placed over rows of beets just after planting. Beginning the early part of January, insects captured by sweeping badly diseased beets with

insect nets were segregated (except in one cage to which field sweepings were added) on the basis of species, and each species selected for test was placed in a separate cage containing healthy beets. The species of insects [4] tested were *Agalliana ensigera* Oman, *Xerophloea viridis* (F.), *Empoasca* sp., *Atanus exitiosus* Beamer,[5] *Melanotrichus* sp., and *Myzus persicae* (Sulz.).

Insects were added to the cages at different times during the next 3 weeks. Some were placed in the cages immediately after being captured, but others were allowed to feed 24 hours on diseased beets before being placed in the cages. In further tests, insects were taken from diseased beets and put into small leaf cages attached to leaves of small beets in pots under cloth cages.

Experiments were made also with diseased beets at the Arlington Experiment Farm by means of *Piesma cinerea* (Say), *Myzus persicae*, and *Eutettix tenellus* (Bak.).

A summary of the results obtained from these tests appears in table 4. Diseased plants were observed in all the large cages to which the leafhoppers later identified (*1*) as *Atanus exitiosus* were added. No infection was obtained on any of the smaller beets to which small cages containing insects were attached.

TABLE 4.—*Transmission tests with yellow wilt of sugar beets by means of insects from field beets in the Rio Negro Valley and from diseased beets in the greenhouse*

Insect	Results with plants in field cages		Results with plants in pots	
	Cages into which insects were introduced	Cages in which plants became diseased	Plants exposed to insects	Plants infected
	Number	*Number*	*Number*	*Number*
Agalliana ensigera	2	0	8	0
Xerophloea viridis	2	0	9	0
Empoasca sp.	1	0		
Atanus exitiosus	2	2	8	0
Myzus persicae	1	0	11	0
Melanotrichus sp.	1	0		
Atanus exitiosus plus additional content of net from sweeping diseased beets	1	1		
Piesma cinerea [1]			10	0
Myzus persicae [1]			10	0
Eutettix tenellus [1]			15	0

[1] Tests made in a quarantine greenhouse at the Arlington Experiment Farm, Arlington, Va., with insects that had fed on diseased beets.

Symptoms of disease were noted first on one of the plants in the cage in which insects swept from diseased beets were placed. The insects, known to include some individuals of *Atanus exitiosus*, were placed in this cage on January 20. On February 19 one of the plants

[4] The authors are indebted to P. W. Oman, of the Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, for identification of the insects used in these experiments.

[5] P. W. Oman, in viewing the specimens of this insect, reported that they represented a new species apparently closely resembling *Atanus dentatus* (Osb.) described in 1923 from Bolivia (*6, p. 57*) and noted also as occurring in northern Argentina (*5*). In the absence of Dr. Oman in military service, C. F. W. Muesebeck kindly arranged to have the collections studied by a specialist in this group. R. H. Beamer (*1*) has described the leafhopper as a new species, *A. exitiosus*.

was wilted and the lower leaves were dead. By March 18 this plant had produced new leaves which were yellow and necrotic and showed definite signs of the yellows phase of the disease. One plant (fig. 8) to which only *A. exitiosus* was added was observed to have symptoms of disease on March 18. At this time the plant had wilted lower leaves, but the central leaves were erect, yellow, and necrotic.

The results obtained in the insect transmission tests, although somewhat limited in scope, are considered as reasonably strong evidence that the leafhopper identified as *Atanus exitiosus* is the vector of the virus of yellow wilt.

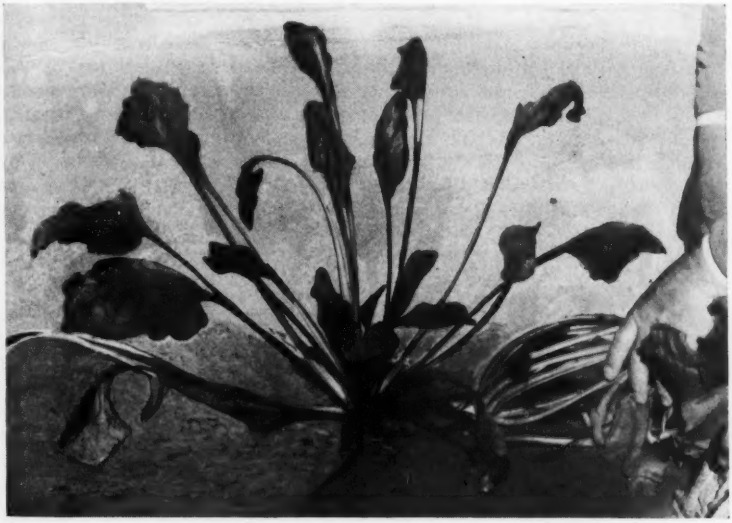

FIGURE 8.—Sugar-beet plant, inoculated by means of the leafhopper *Atanus exitiosus*, showing yellow, necrotic leaves.

THE LEAFHOPPER SUSPECTED AS VECTOR

Atanus exitiosus, which probably is the vector of the virus of yellow wilt of sugar beets in Argentina, was found during January 1941 generally distributed in the valley of the Río Negro throughout the region where sugar beets were grown. In spite of this general distribution, however, it was not abundant in any of the sugar-beet fields.

In shape and size *Atanus exitiosus* is similar to *Eutettix tenellus*, the vector of the virus of sugar-beet curly top. The dorsal side is light brown, and the ventral side has a distinctly yellowish cast. The males are somewhat smaller than the females. In field cages the leafhopper deposited eggs in beet leaves singly or in groups just beneath and parallel to the surface of the epidermis. Eggs were clearly visible under low magnification. They hatched into light-colored nymphs in about 12 days, and the nymphs appeared to thrive on sugar beet.

From the observations made in the field it seemed probable that sugar beet may be one of the more favorable host plants of *Atanus*

exitiosus, at least among the cultivated crop plants. In spite of the fact that the insect bred readily on sugar beets under cages, however, extensive search revealed no nymphs on beets outside the cages. This failure to find nymphs indicates clearly that the insect does not build up large populations on sugar beet, possibly owing partly to a late migration of leafhoppers into the fields and a low population of migrant adults.

Although *Atanus exitiosus* appeared to be more numerous on sugar beet than on any other plant, it was captured also on alfilaria (*Erodium cicutarium* (L.) L'Hérit.) and on Russian-thistle (*Salsola kali* L.). Two unidentified species of mustard (*Brassica*), an unidentified species of *Chenopodium*, and *C. album* were abundant in and adjacent to several of the beet plantings, but no leafhoppers were taken from any of these plants. Potato and tomato plantings seemed free of this leafhopper. Extensive sweepings of desert vegetation of various kinds and at different distances from the beet plantings were made, but no leafhoppers of this species were captured except in a few instances in the immediate vicinity of beet plantings. It seems certain that during January 1941 none of the native vegetation of the area where beet plantings were located was harboring more than a very small population of *A. exitiosus*.

THE CAUSAL AGENT

The evidence presented in this paper appears ample to justify the conclusion that the yellow wilt prevalent in beet plantings since 1929 in the lower Río Negro Valley is caused by a virus. On the basis of the probable insect vector and the symptoms produced on known susceptible hosts, the virus clearly belongs in the genus *Chlorogenus* established by Holmes (*3*). The name *Chlorogenus patagoniensis* is proposed for this virus, with the following description.

Chlorogenus patagoniensis n. sp.

Produces destructive disease on sugar beet (*Beta vulgaris*), characterized by sudden wilting and death of affected plants or by stunting, yellowing, and stimulation of axillary bud development. Vein yellowing sometimes present on affected plants. Transmissible by graft and by means of two species of dodder (*Cuscuta subinclusa* and *C. campestris*), but not by juice inoculation. Probable vector a leafhopper identified as *Atanus exitiosus* Beamer.

Descriptive habitat: Valley of the lower Río Negro in Argentina.

If the classification and nomenclature of Smith (*7*) are used, the virus should be designated *Beta virus* 6.

POSSIBLE METHODS OF CONTROL

It has been found that if sugar-beet plantings are made after the middle of December in the Río Negro Valley, they escape yellow wilt in large measure. This date of planting is later than that which gives maximum yields under disease-free conditions, but it is possible that sugar beets could be produced on a profitable commercial scale by taking advantage of the failure of the disease to develop extensively in late-planted beets.

It is not known whether the disease can be partly controlled by reducing the host plants on which the insect vector breeds. There are certain factors, however, that do not preclude the possibility of beneficial results from sprays that markedly reduce the vector popula-

tion. Each season, a certain percentage of the plant population has survived free of all symptoms of disease. It seems probable that these plants escaped infection because the vector was not sufficiently abundant to infect all the plants. If this is the correct explanation for the presence of healthy plants in affected fields, any reduction in the population of the insect vector before the insects have had an opportunity to transmit the virus would be expected to reduce correspondingly the amount of disease.

No evidence has been obtained that any variety of sugar beet has any appreciable resistance to yellow wilt. Kleinwanzleben brands have been used for most of the plantings in the Río Negro Valley. All importations of these brands of sugar beet have proved to be about equal in susceptibility. In 1938–39, 20 European varieties and 2 varieties from the United States (U. S. 12 and U. S. 33) were tested for relative susceptibility in variety test plots near Conesa. The disease was very severe, and large percentages of plants of all varieties were affected. There was no evidence, however, that any one of these varieties was superior to the others in resistance. The test was repeated in 1939–40 with 28 European varieties, U. S. 12, and U. S. 33; the results were similar to those obtained the previous season.

Efforts were made also, through selection and propagation of plants that remained healthy in severely diseased fields, to obtain a variety resistant to yellow wilt. In 1938–39 more than a thousand roots from apparently healthy plants were selected from plantings from seeds of Kleinwanzleben (German), Udycz (Polish), Cesena (Italian), Vilmorin (French), and varieties of Swedish and Danish origin, in fields near Conesa in which not more than 10 percent of the plants had survived. The selected roots were stored during the winter and planted out the following spring for seed production. Seeds from these selections were planted in variety test plots in 1940. The progenies showed no greater resistance, however, than that possessed by the varieties from which the selections were made.

The apparent lack of any appreciable variability in resistance among the many sorts of sugar beets tested and the fact that progeny from individual plants that survived in badly diseased fields showed no increase in resistance over the parent material is discouraging from the standpoint of selection and breeding for disease resistance. In every field in which this disease has occurred there have been in general two types of plants: (1) Those that showed no effects of the disease and (2) those that died of the disease or that were so severely injured that they would not produce seeds. These two types of plants either represent immunity and extreme susceptibility or else the apparently healthy plants merely escaped infection. The latter condition appears to be the more probable, and it is likely that selections as made did not deal with resistant plants but with those that had escaped infection. From information now available, susceptibility to yellow wilt in sugar beets seems to present a condition similar to that found with respect to susceptibility to curly top in tomato, in which effective factors for resistance have not been discovered. It seems probable from the evidence now available that it may be very difficult to develop varieties resistant to yellow wilt from commercial material.

DISCUSSION

The destructive attacks on sugar beets when they were introduced into the valleys of the Río Colorado and the Río Negro in Argentina are typical of what has occurred in a number of other instances in which an agricultural crop came in contact with a new pathogen under changed environmental conditions. It seems probable that there exists in the native vegetation of this part of Argentina a virus, transmitted by a local insect, which is capable of causing only mild symptoms or no symptoms on the native plants that harbor it but which has proved to be extremely severe on introduced sugar-beet plants.

The diversity of symptoms produced on sugar beet has led to speculation as to whether more than one causal agent is involved. Results of observations and experiments strongly support the concept that all the different types of symptoms observed are the result of the action of a single causal agent and that this agent is a virus. That all symptoms described result from attack by the same causal agent is indicated under field conditions by the presence of plants that first wilt and then recover to an appreciable degree and produce yellow leaves, sometimes with the stimulation of growth in axillary buds. The fact that yellowed and wilted plants selected in the field and transferred to the greenhouse all produced yellowed plants with an abundant development of axillary buds supports the field evidence. Furthermore, transmission by graft from plants of the two types gave only the typical yellowing and bunchy tops characteristic of the disease under greenhouse conditions.

The factors involved in the production of the widely dissimilar symptoms characteristic of the yellows and wilt phases of yellow wilt, respectively, are not known definitely, but certain observations that appear to have a bearing on the subject furnish a basis for speculation. It seems significant that only the yellows phase of the disease has been produced in the greenhouse and that in the field this phase has appeared early in the season, followed later by the wilt phase. These facts suggest that the type of symptoms produced is determined largely by environmental conditions to which the plants are subjected after infection.

It seems probable, as is known to be true for a number of other virus diseases, that one of the first effects of the disease is the production of a severe initial shock in the infected plant. This may involve sudden extensive injury to the rootlets, resulting in drastic reduction in the ability of the plant to take up water from the soil, or it may involve changes that result in the reduction of the ability of the plant to transport water. If the onset of the disease occurs in the greenhouse, where atmospheric humidity is high, or if it occurs early in the season in the field, when the days are cooler and transpiration rates are low, the plant may be able to survive the initial attack, partially recover, and establish a condition in which it is able to grow sufficiently to produce the yellows phase of the disease. However, in the field, if the attack comes later in the season, when the plants are larger and when transpiration rates are increased by high temperatures and strong winds such as are common in the Río Negro Valley, transpiration rates may be so high that the water content in much of the plant may drop below the critical wilting point during the period of initial shock,

and death of a part or all of the plant may result before the plant is able to make the adjustments necessary for partial recovery from early stages of attack.

Even though yellow wilt occurs in a part of the world where the beet-sugar industry is not yet of major importance, this disease has tremendous significance on account of its potentialities for destruction if introduced into other beet-sugar-producing areas of the world. The fact that the disease has, so far as knowr, not yet spread to the beet sections of Uruguay and to the more humid areas of Argentina may indicate that the vector is restricted to a relatively arid climate. The climatic conditions in the Río Negro Valley are very similar to those in large sections of the western part of the United States, where any insect vector able to thrive in the Río Negro Valley might also do well. Therefore, if both vector and virus were introduced into the West, there is reason to believe tbat the sugar-beet industry might face a major catastrophe. The threat is even more menacing because of the fact that it is reasonably certain that none of the more important varieties at present grown in the United States is resistant to the disease. There is evidence also that the sugar beet possesses almost no resistance to this disease. Development of varieties resistant to it would be very difficult, and success could be hoped for only after long and difficult work of selection and breeding. A somewhat parallel problem confronted the workers on the virus disease curly top in the Western States, but persistent tests over a period of years eventually yielded varieties with a very high degree of resistance to the disease. Whether this outstanding success from selection and breeding for curly top resistance could be repeated with yellow wilt probably depends on whether breeding material could be found that has a greater degree of resistance than has any of the varieties of sugar beets so far tested.

SUMMARY

A disease for which the name "yellow wilt" is suggested has caused severe damage to sugar beets in the valley of the Río Negro in Argentina, probably since the industry was first established in that region in 1929.

One phase of the disease is characterized by stunting, yellowing of foliage, slight stimulation of development of axillary buds, and leaf malformation; wilting and rapid killing of plants are characteristic of another phase. Plants are attacked usually after they attain considerable size.

Under greenhouse conditions, the disease was transmitted by grafting diseased to healthy tissue and by each of two species of dodder (*Cuscuta subinclusa* and *C. campestris*), but not by juice inoculation. Field observations indicate that a leafhopper (*Atanus exitiosus* Beamer) is the vector of the causal agent.

The evidence indicates that yellow wilt is caused by a previously unknown virus. The designations *Chlorogenus patagoniensis* n. sp. and *Beta virus* 6 are proposed for the virus in accordance with the systems of classification and nomenclature of Holmes (*3*) and Smith (*7*), respectively.

In extensive tests in 2 seasons, involving 20 European varieties and 2 varieties from the United States the first season and 28 European

varieties and 2 varieties from the United States the second season, no evidence was obtained of an appreciable amount of resistance in any variety of sugar beet.

Satisfactory control measures for the disease have not been developed. Plantings made after the middle of December escape infection in the Río Negro Valley, but this date of planting is too late for maximum yields. Selection and propagation of healthy beets from badly infested fields have not resulted in increased resistance in the progeny. Other possible methods of control have not been tested.

LITERATURE CITED

(1) Beamer, R. H.
1943. A new Atanus from Argentina, South America (Homoptera-Cicadellidae). Wash. Ent. Soc. Proc. 45: 178–179, illus.

(2) Henderson, C. F.
1928. Exploration in the Argentine Republic for parasites of the beet leafhopper, Eutettix tenellus (Baker). Jour. Econ. Ent. 21: 863–871, illus.

(3) Holmes, F. O.
1939. Handbook of phytopathogenic viruses. 221 pp. Minneapolis, Minn.

(4) Huyskes, J. A.
1939. Physiologische ziekten van de suikerbiet, veroorzaakt door foutieve bevloeiing. Tijdschr. over Plantenziekten 45: 204–223, illus.

(5) Oman, P. W.
1936. A generic revision of American Bythoscopinae and South American Jassinae. Kans. Univ. Sci. Bul. 24: 343–420, illus.

(6) Osborn, H.
1923. Neotropical Homoptera of the Carnegie Museum. Pittsburg, Carnegie Inst. Mus. Ann. 15: 8–79, illus.

(7) Smith, K. M.
1937. A textbook of plant virus diseases. 615 pp., illus. Philadelphia.

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